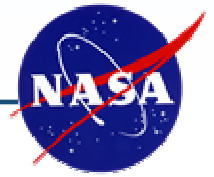
Microfluidic Biochip Design

Dr. Charles Panzarella

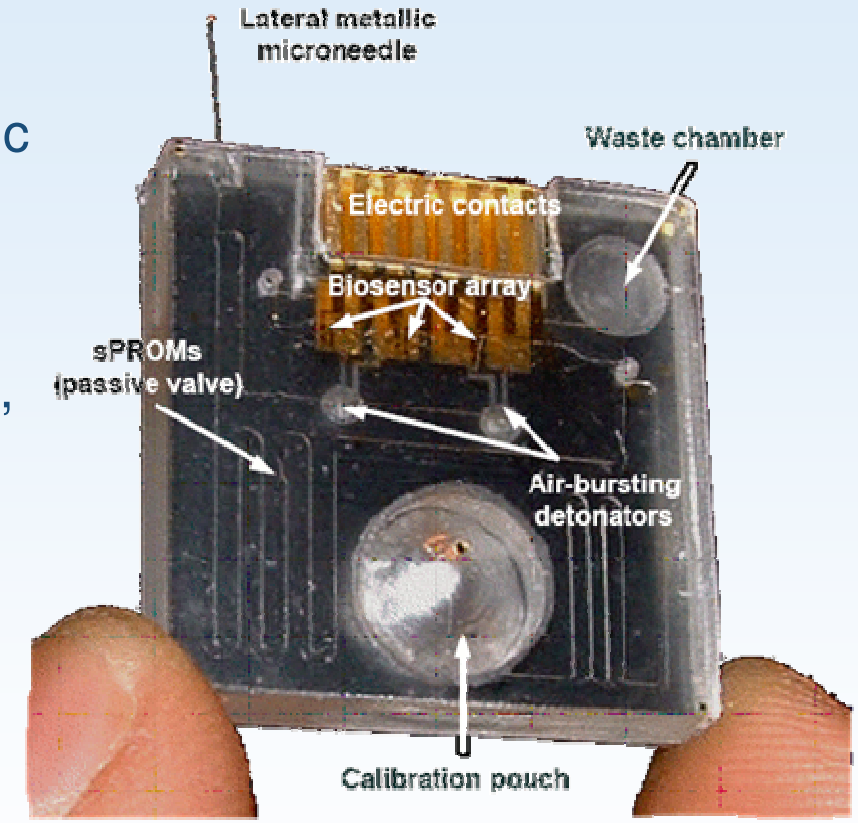
PI: Arnon Chait

Co-PIs: Charles Panzarella, David Jacqmin, Emily Nelson, Vladimir Pines, Marianne Zlatkowski, Maria Kuczmarski, Mohammad Kassemi



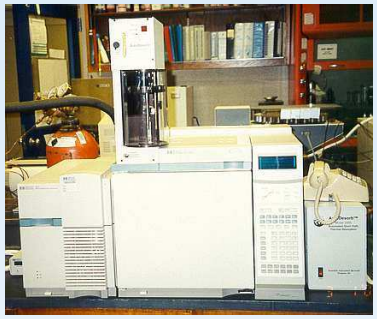
What is a Biochip?

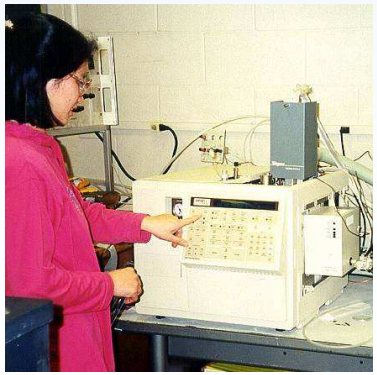
- Miniaturized microfluidic/nanofluidic instrument for performing multiple biological assays (tests).
- Consists of micron-sized channels, valves, pumps, mixers, reactors, and biosensors.
- Promises to revolutionize medical diagnostics when it fully matures.



* Chong H. Ahn, University of Cincinnati

Why Use Biochips?

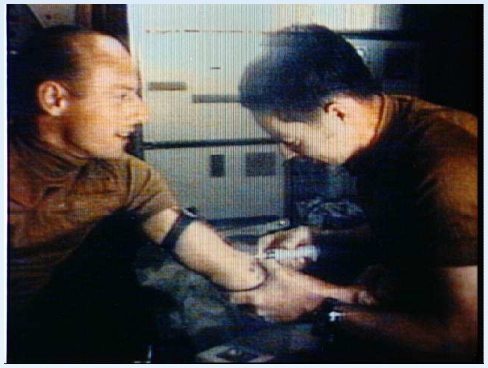




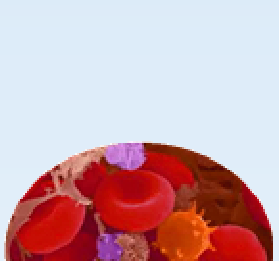
Nanofluidic Lab-on-a-Chip

These commercialized versions of ORNL's Lab-on-a-Chip are products of Caliper Technologies, Inc. and Agilent Technologies, Inc.

Why Does NASA Need Biochips?

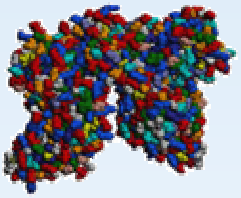


In-Flight Blood, Saliva, Urine Tests



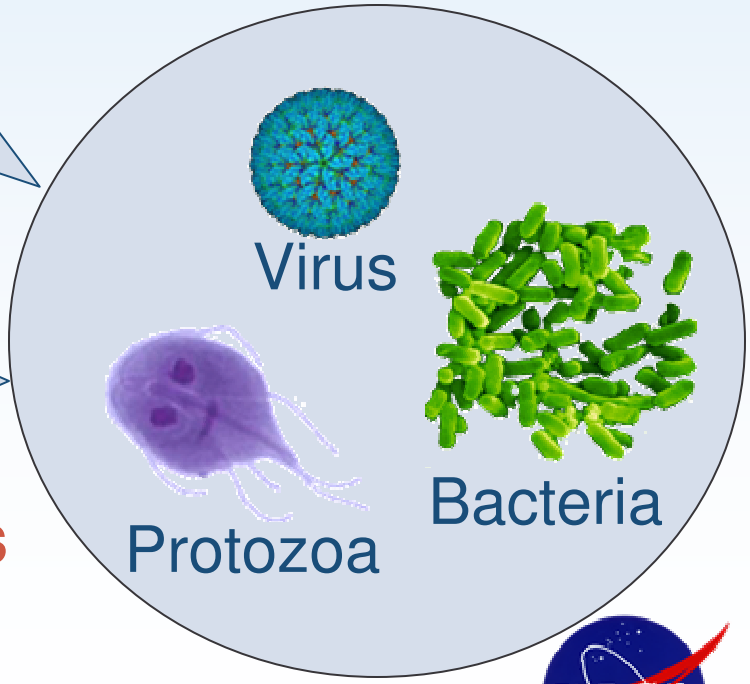
Genetic

Cell Count



Chem-20

Test Water/Food/Air/Surfaces for Contamination



NASA's Requirements

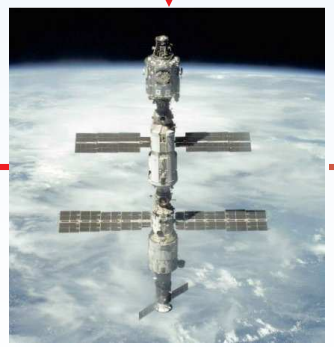
Sample Preparation

Collection

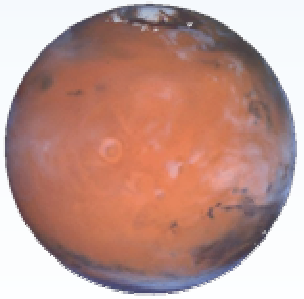
Integrated, Portable Device

Detection

Moon



Mars

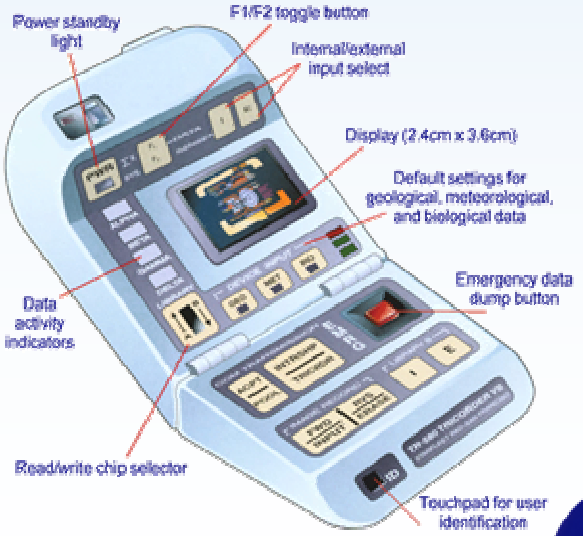


Space Station

General Design Requirements

- All components must be integrated into one small, lightweight, compact device (handheld or tabletop).
- Must be completely contained; no external sample preparation.
- Must be fully automatic with minimal operator intervention.
- Use small amounts of reagents, sample fluids and energy.
- Modular and reusable (multiple, disposable plug-in cartridges).
- Must work over extended periods of time without maintenance (e.g. trip to Mars and back).
- Detect multiple pathogens at once.

For the Star Trek fans out there, think **Tricorder**:

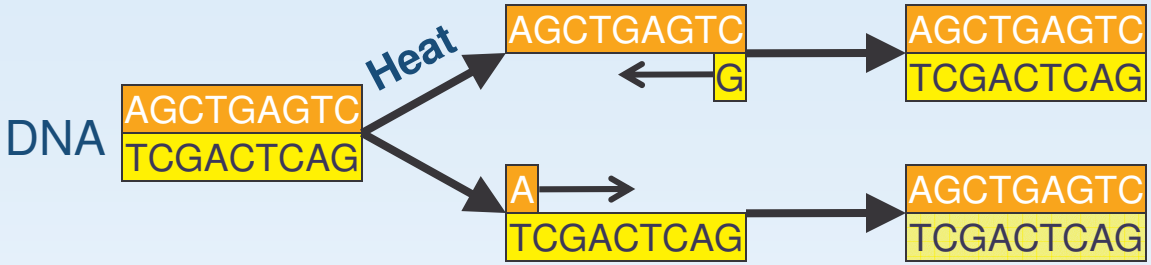


Pathogen Detection Objectives

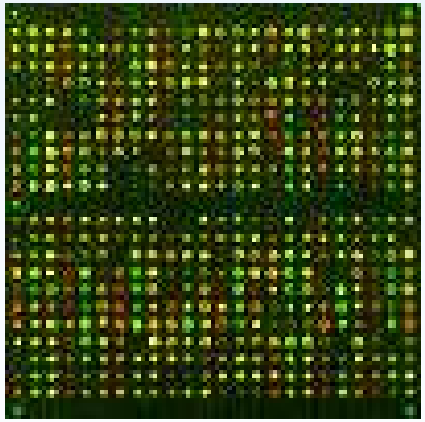
Objective	Approach
1. **Presence/Absence**	Look For Pathogen DNA
2. **Enumeration**	Measure Quantity of DNA
3. **Viability**	Look For Presence of RNA

Nucleic Acid Analysis *Could* Accomplish All These Objectives

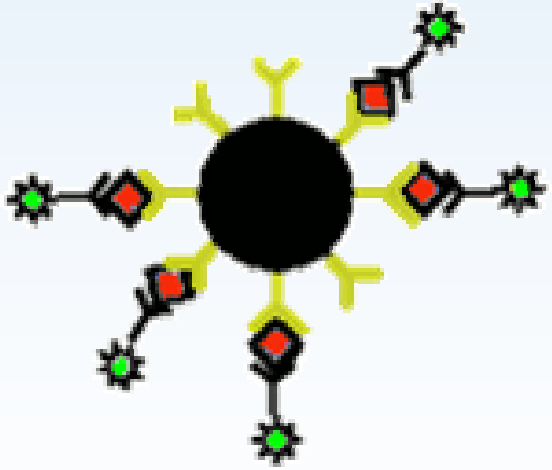
Some Methods of Pathogen Detection



Real-Time PCR

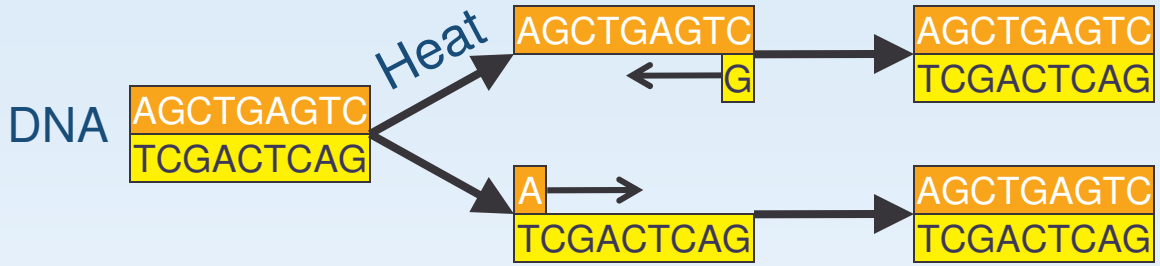


DNA Microarray



Microbead Immunoassay

Real-Time PCR

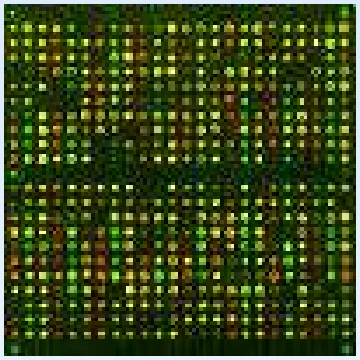


Process repeated 20-30 times

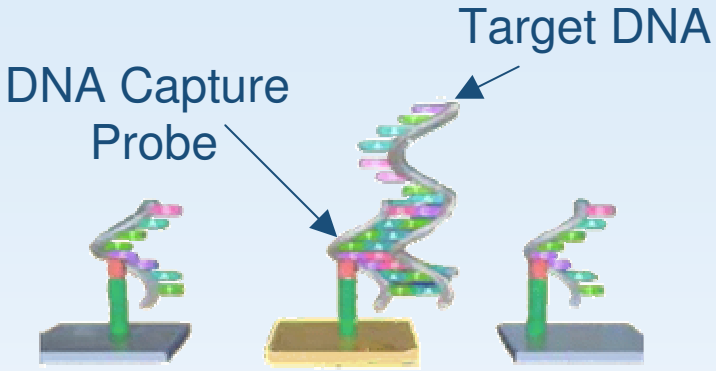
Polymerase Chain Reaction (PCR) Amplification

- Logarithmic amplification of specific regions of DNA.
- Choose regions specific to a particular pathogen (with primers).
- Fluorescent signal measured during the reaction.
- Fluorescent intensity proportional to amount (if any) of DNA.
- Doesn't require electrophoresis or gels like traditional PCR.
- Faster, quantitative and more sensitive than traditional PCR.

DNA Microarray



DNA Microarray



Solid-Phase Hybridization

- Short single DNA strands (oligos) representative of the pathogen are attached to a solid substrate.
- Unknown target DNA passes over and hybridizes (zips up) if they match.
- Hybridization detected by fluorescence (or electrical signal).
- Massively parallel - can test for multiple pathogens at once.
- PCR amplification can still be used to increase sensitivity.

What Systems Available Now (Soon)

Real-Time PCR

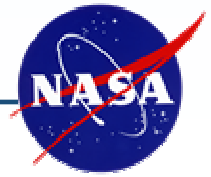
- Lawrence Livermore National Laboratory – Handheld Advanced Nucleic Acid Analyzer (**HANAA**)
- Agilent/Caliper – **Bioanalyzer**

DNA Microarray

- Motorola – **eSensor**
- Pacific Northwest National Laboratory – Biodetection Enabling Analyte Delivery System (**BEADS**)

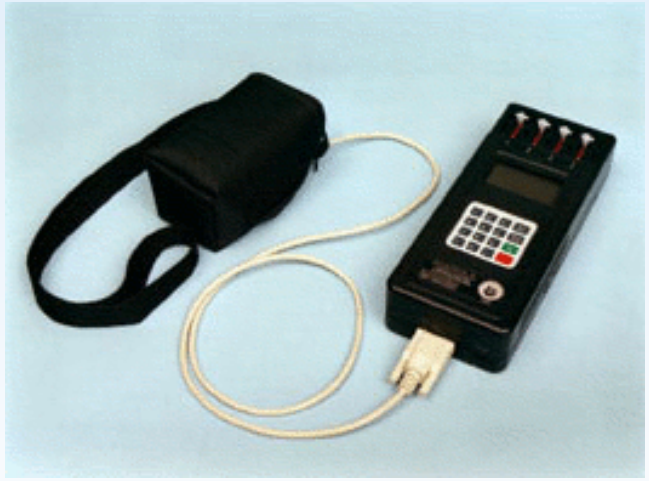
Microbead Immunoassays

- Lawrence Livermore National Laboratory - Autonomous Pathogen Detection System (**APDS**)



LLNL - HANAA

Handheld Advanced Nucleic Acid Analyzer (HANAA)



- Size of a brick, weighs about 2 lbs.
- Detect as few as 10 bacteria in 0.01 mL of liquid (< 30 minutes).
- Used in 2002 by UN weapons inspectors in IRAQ.
- Still requires operator intervention to prepare sample fluid (mix with buffer and chemicals).

LLNL - APDS

Autonomous Pathogen Detection System (APDS)

- Bio ”smoke detector.”
- Completely automated.
- Aerosol sampling, in-line sample preparation, multiplex flow cytometer detection and identification assays, and orthogonal flow-through PCR amplification and detection.
- Measure up to 100 different agents and controls in a single sample.
- Not very small or portable.

Agilent 2100 Bioanalyzer

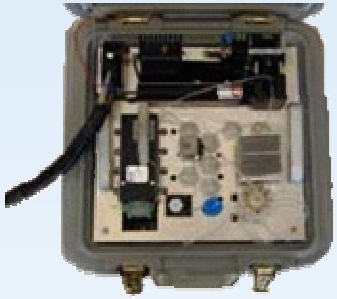
- Tabletop system with interchangeable LabChip cartridges developed by Caliper.
- LabChip kits for RNA, DNA, Protein, Cell analysis.
- Integrates sample handling and detection.
- Still requires some external sample preparation.
- Uses pipette for sample loading.

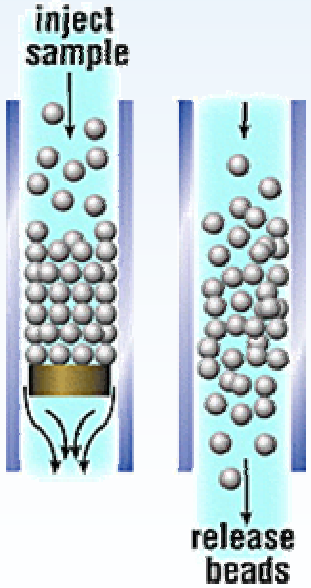
Motorola - eSensor

- DNA microarray detector (multiple pathogens at once).
- Electronic detection of DNA hybridization.
- Smaller and cheaper than standard optical methods.
- Still requires some manual sample preparation.

PNNL - BEADS

Biodetection Enabling Analyte Delivery System (BEADS)





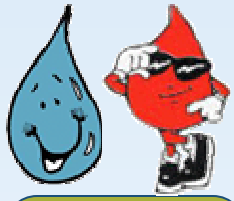
- Sophisticated sample preparation system.
- Uses specific microbeads to isolate and concentrate bacteria, spores, viruses and their DNA from air, dirt or water samples.
- Uses DNA microarray for detection.
- Fully autonomous.
- Results from testing can be sent to remote locations.

Microgravity Issues

- Sample collection must be fully automated, integrated and not rely on gravitational forces (no pipetting).
- Entire system must be contained – guaranteed sealing of biochip before/during/after use to avoid contamination.
- Bubbly sample fluids may need to be filtered.
- Gene expression known to be different in microgravity - may affect performance of mRNA based microarrays.
- Durability – must withstand accelerations and vibrations due to launch and descent.
- Longevity - must continue to work over long periods of time (possibly years) without maintenance.

Must anticipate all microgravity effects beforehand – traditional empirical iteration not possible.

All Stages Must Be Integrated

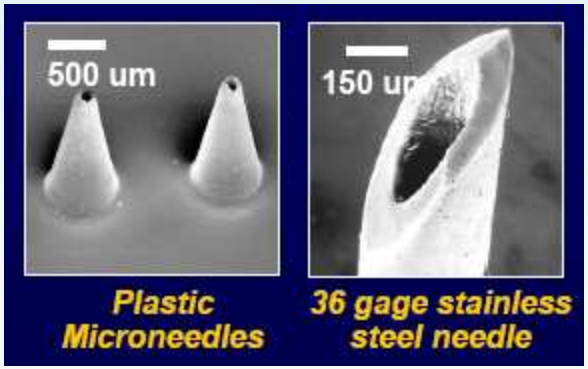


Sample Collection → Sample Filtering → Lysis → Nucleic Acid Purification → Target Amplification → Detection And Analysis

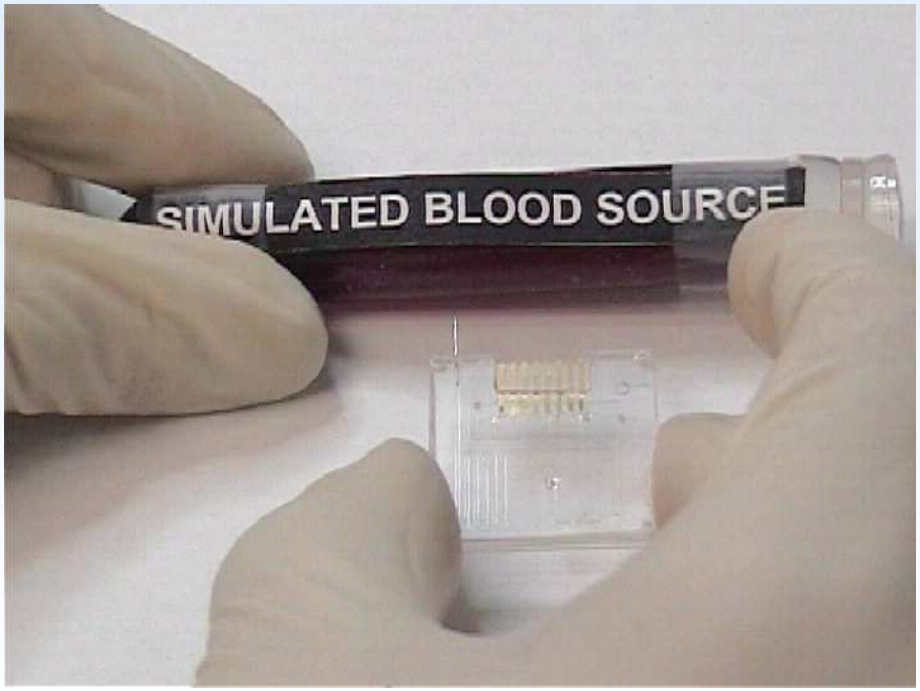
Most Difficult

Sample Collection

Sample Collection → Sample Filtering → Lysis → Nucleic Acid Purification → Target Amplification → Detection And Analysis



Microneedles



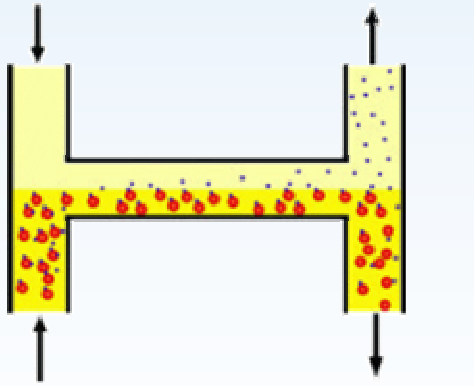
Wicking action is often sufficient*

* Chong H. Ahn, University of Cincinnati

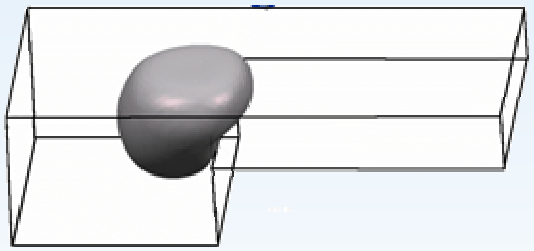
Sample Filtering

Sample Collection → **Sample Filtering** → Lysis → Nucleic Acid Purification → Target Amplification → Detection And Analysis

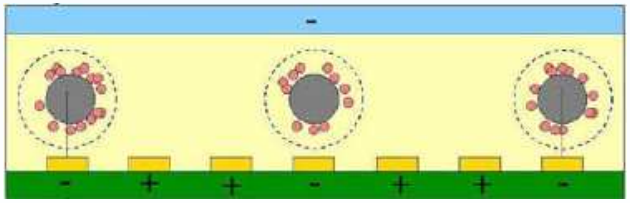
Most significant hindrance to an integrated device



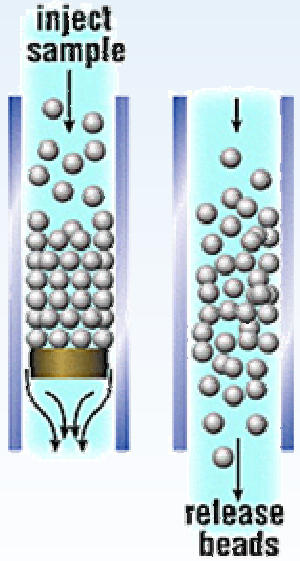
Diffusive Separators



Bubble Filters

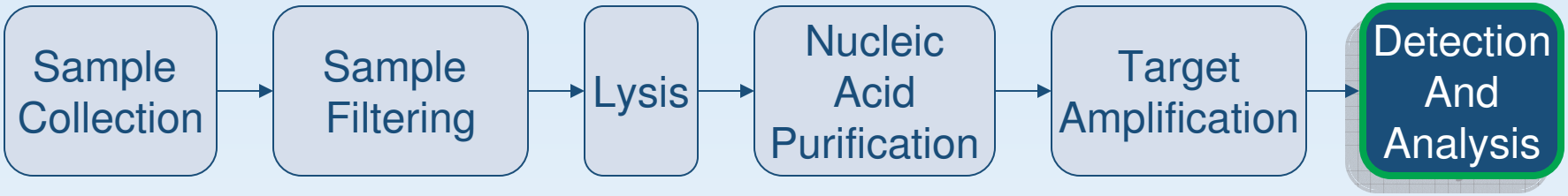


Cell Manipulation (DEP)



Microbead Collectors

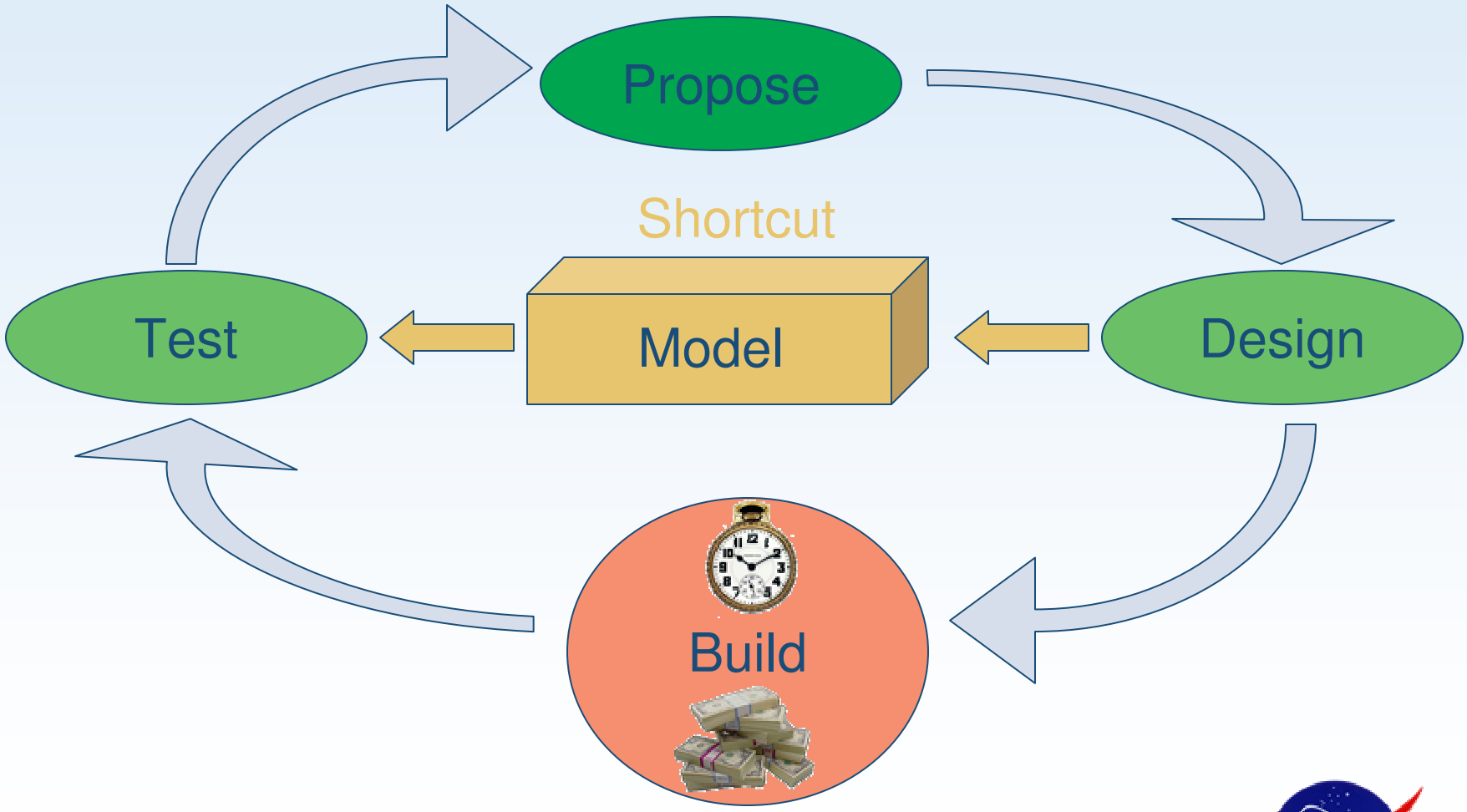
Detection and Analysis



- Not entirely clear yet how microgravity will effect the operation of nucleic acid based detection methods (bubbles, radiation, different genetic expression).
- Generally need increased sensitivity and selectivity.
- Detection method must remain viable for long periods of time without maintenance.
- Must include genetic signatures for all pathogens of interest.

How Model Simulations Can Help

Iterative Design Cycle



Why Biochip Simulations?

To assure optimal, error-free operation in Space out-of-the-box without iterative cycling:

- Ground-based iterative design does not account for unique microgravity environment.
- Must get it right first time around.
- Cannot change the design once its on its way to Mars.

"In our previous studies, the microchip flow chamber was based on a pragmatic approach in the absence of computational or modeling tools for microchip microfluidic design. With the rapid development of computational fluid dynamic simulation, this type of software would be an ideal tool for microchip design." – Yuen et al. 2001 *Microchip Module for Blood Sample Preparation and Nucleic Acid Amplification Reactions.*

Our Contribution

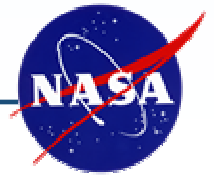
1. **Transfer of commercial biochip technology to NASA.**
   - Surveying commercial products to find those that most closely meet NASA's needs.
   - Work with companies and other research labs to adapt their technology for autonomous, long-term microgravity use.
   - Offer our expertise in microfluidics and microgravity phenomena to guide design modifications.
2. **Biochip Modeling.**
   - Develop computational models of critical components and complete systems to optimize their design.
   - Use models to explore alternative concepts that may improve sensitivity and specificity.
   - Propose and lead a space experiment to test a prototype device.

Focus of Our Modeling Efforts

1. **Filtering/Separation:**
   - Thorough evaluation/optimization of existing filtering methods.
   - Looking at more novel filtering/collection methods (microbeads).
   - Fundamental theoretical work on systems of interacting particles.
2. **Detection/Analysis:**
   - Evaluate different detection methods (optical, electrical, chem.)
   - Optimization of hybridization/immunoassay techniques.
   - Reevaluation of fundamental kinetics of hybridization process.
3. **System level flow model of integrated device:**
   - Multi-phase fluid flow through all connected biochip components.
   - Evaluate different pumping/mixing methods.
   - Parametric/optimization studies for all components.

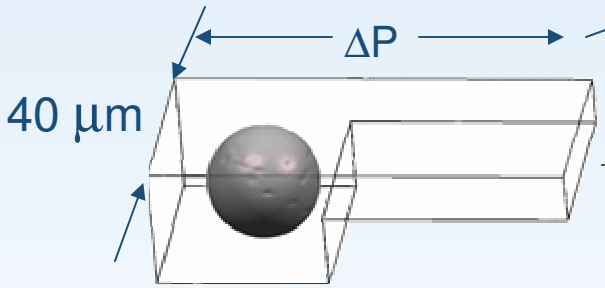
Several Examples of Biochip Modeling

1. **Bubble/Particle Filter Design.**
2. Particle Manipulation with Dielectrophoresis.
3. DNA Hybridization in a Microchannel.
4. Microbead-Based Immunoassay.
5. Filling by Wicking.



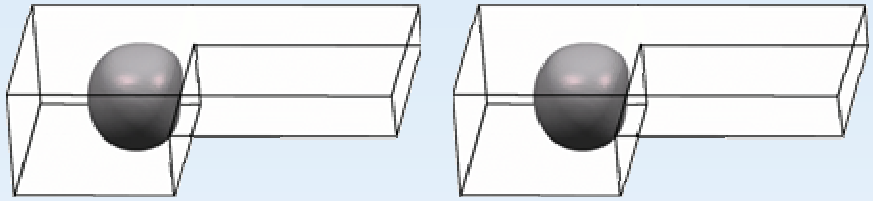
Air Bubble Entrapment / Bubble Filter

VOF simulations with CFD-ACE



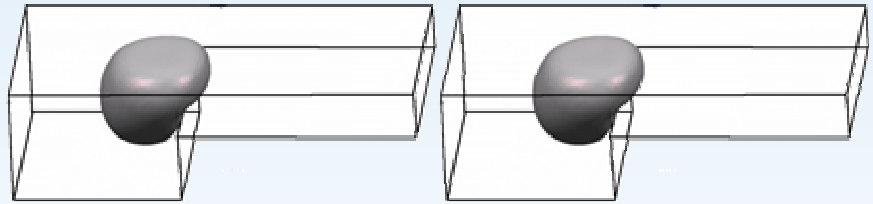
ΔP=5,000 Pa

Air bubble stays in large channel:



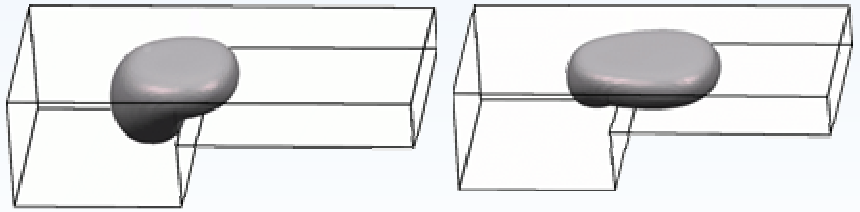
ΔP=10,000 Pa

Air bubble gets stuck in small channel:



ΔP=15,000 Pa

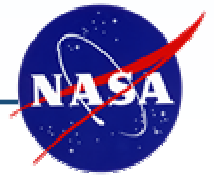
Bubble moves freely into small channel:



How can we filter just the bubbles without removing or damaging the cells?

Several Examples of Biochip Modeling

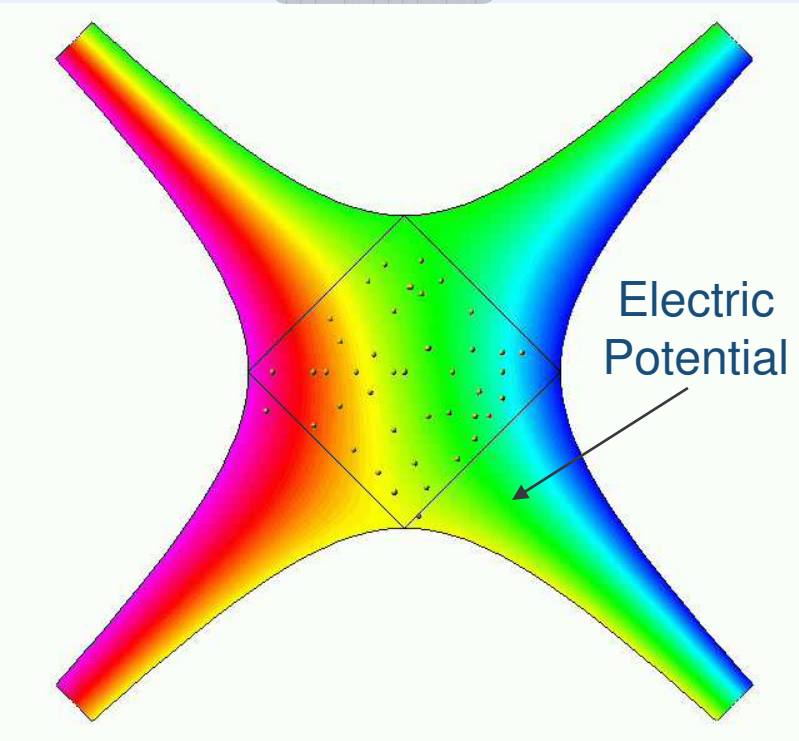
1. Bubble/Particle Filter Design.
2. **Particle Manipulation with Dielectrophoresis.**
3. DNA Hybridization in a Microchannel.
4. Microbead-Based Immunoassay.
5. Filling by Wicking.



Particle Focusing With DEP

- Quadrupole electrode configuration generates rotating electric field.
- Solid particles (5 μm dia.) suspended in water and randomly distributed.
- Repelled into the center by negative DEP force.

Model



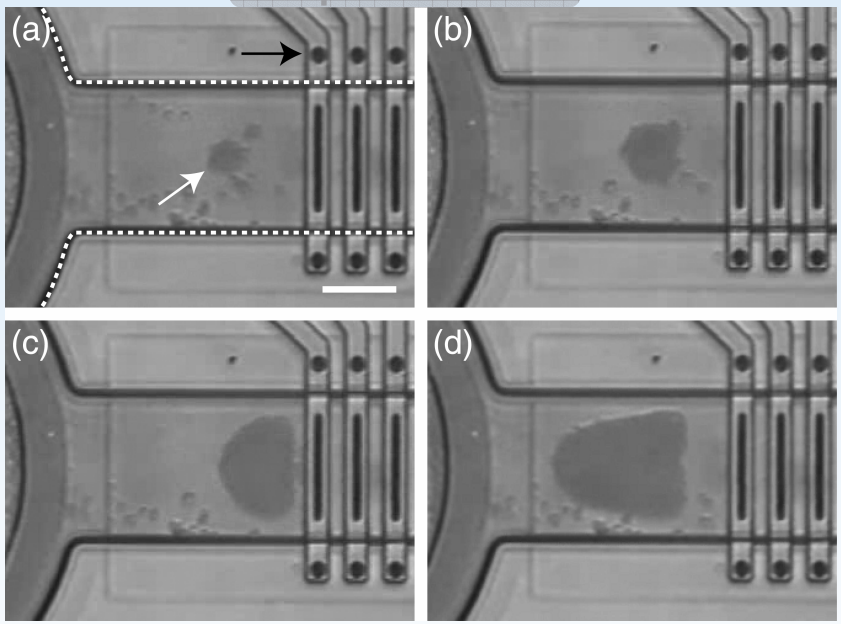
Experiment

Particle Collection With Dielectrophoresis

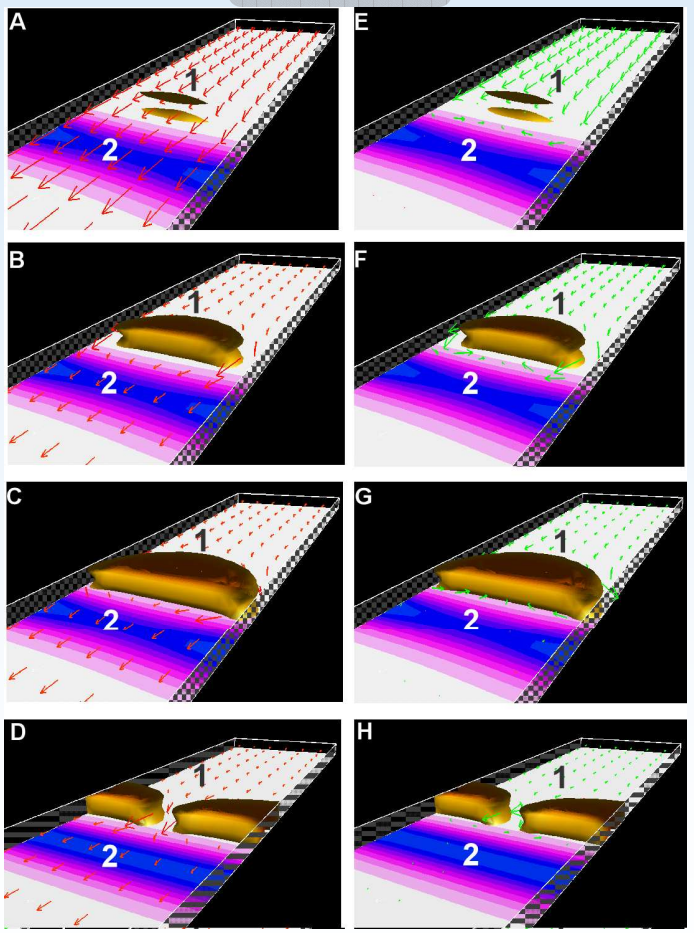
- Modeling of a particle collection device designed and built by Sandia Labs.
- A custom 3D phase-field code was written to calculate particle movement and fluid flow through the device.
- Consists of a microchannel with a high electrical field crosswise electrode that tends to block particles with negative dielectric coefficients.
- Found to be very effective at trapping particles.
- Both experimentally and computationally it was found that particles aggregated into bolus or cylinder shaped forms in front of the electrode gate.

Particle Collection With Dielectrophoresis

Experiment



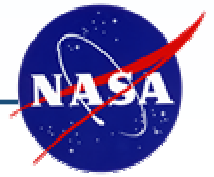
Model



Contributed to the design and development of micro-scale devices for measuring environmental contamination.

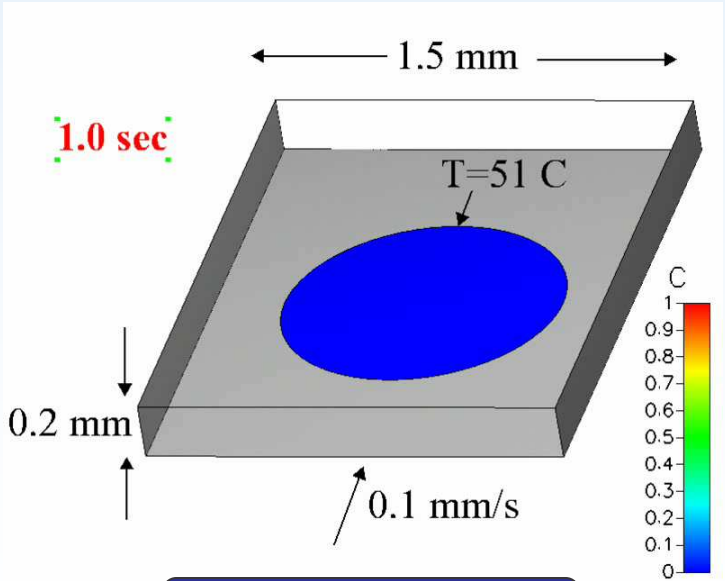
Several Examples of Biochip Modeling

1. Bubble/Particle Filter Design.
2. Particle Manipulation with Dielectrophoresis.
3. **DNA Hybridization in a Microchannel.**
4. Microbead-Based Immunoassay.
5. Filling by Wicking.

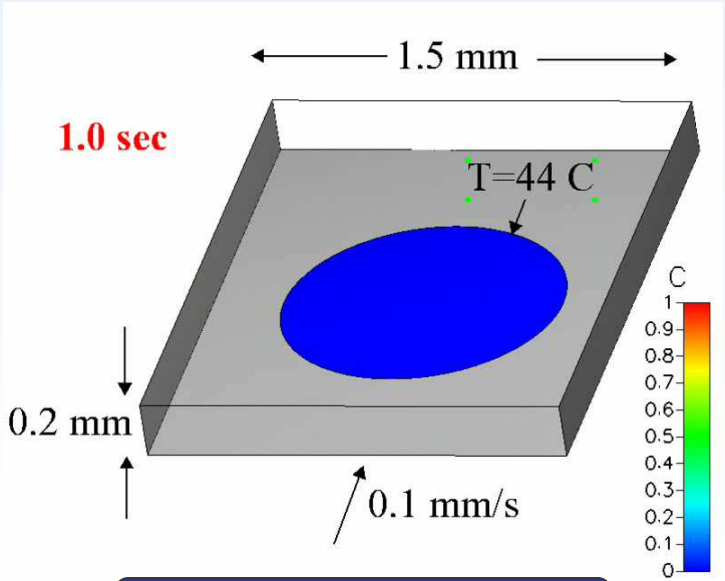


DNA Hybridization in a Microchannel

- Hybridization of dT_{20} probes and dA_{20} targets.
- Melting temperature of dT_{20}:dA_{20} duplex is 51°C.
- With single mismatch, dT_{20}:$d(A_9TA_{10})$ has melting temperature of 44°C.
- Target concentration at the inlet is 0.1 μM, fluid speed is 0.1 mm/s.
- 50°C temperature drop across channel.
- Different spatial hybridization patterns result from mismatch.



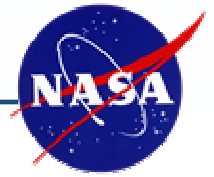
Perfect Match



Single Mismatch

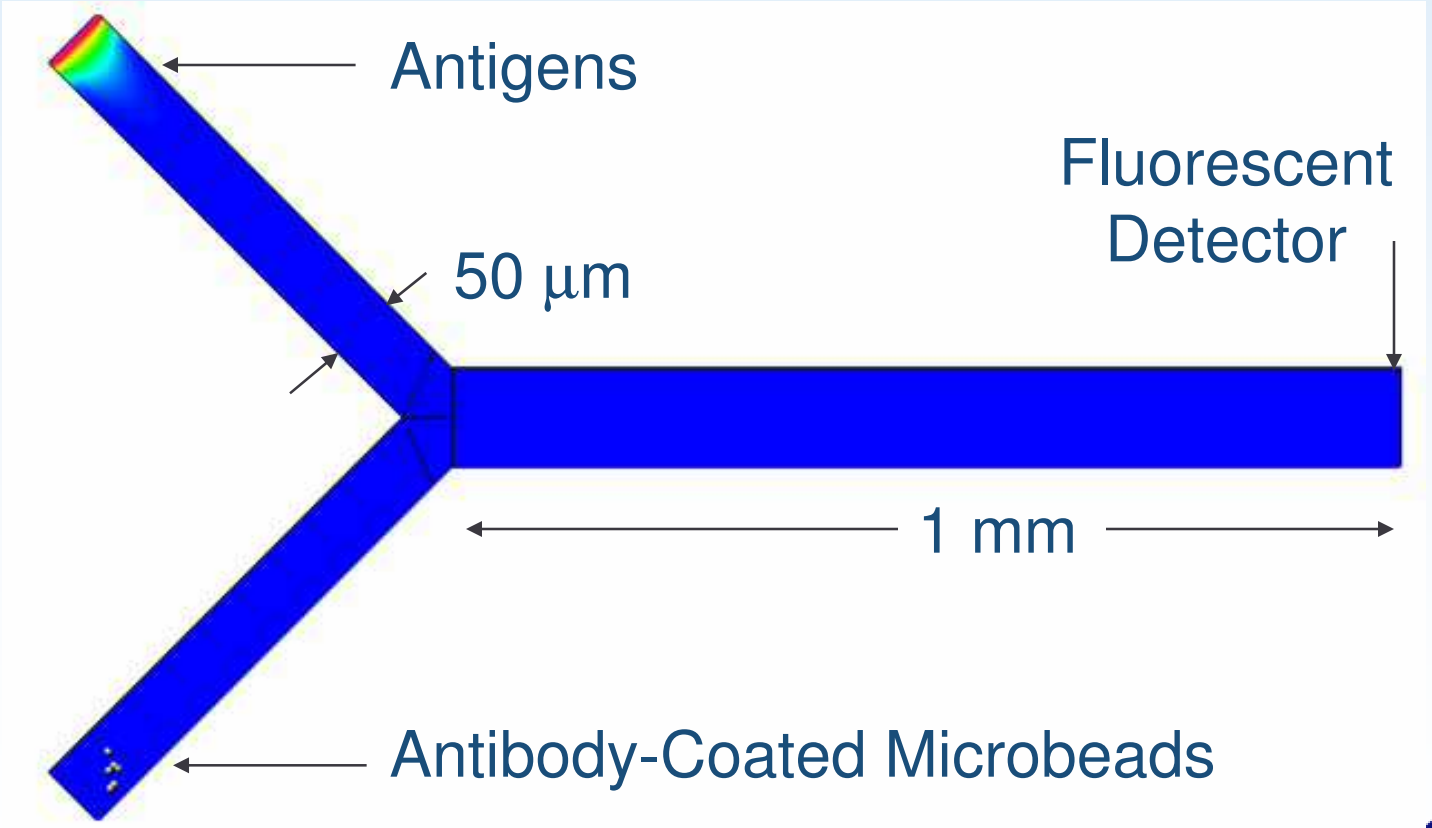
Several Examples of Biochip Modeling

1. Bubble/Particle Filter Design.
2. Particle Manipulation with Dielectrophoresis.
3. DNA Hybridization in a Microchannel.
4. **Microbead-Based Immunoassay.**
5. Filling by Wicking.



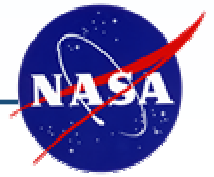
Microbead Immunoassay in a Y-Channel

- Microbeads (5 μm dia.) coated with specific antibodies.
- Mixed with analyte (antigens) in a 1 mm long channel.



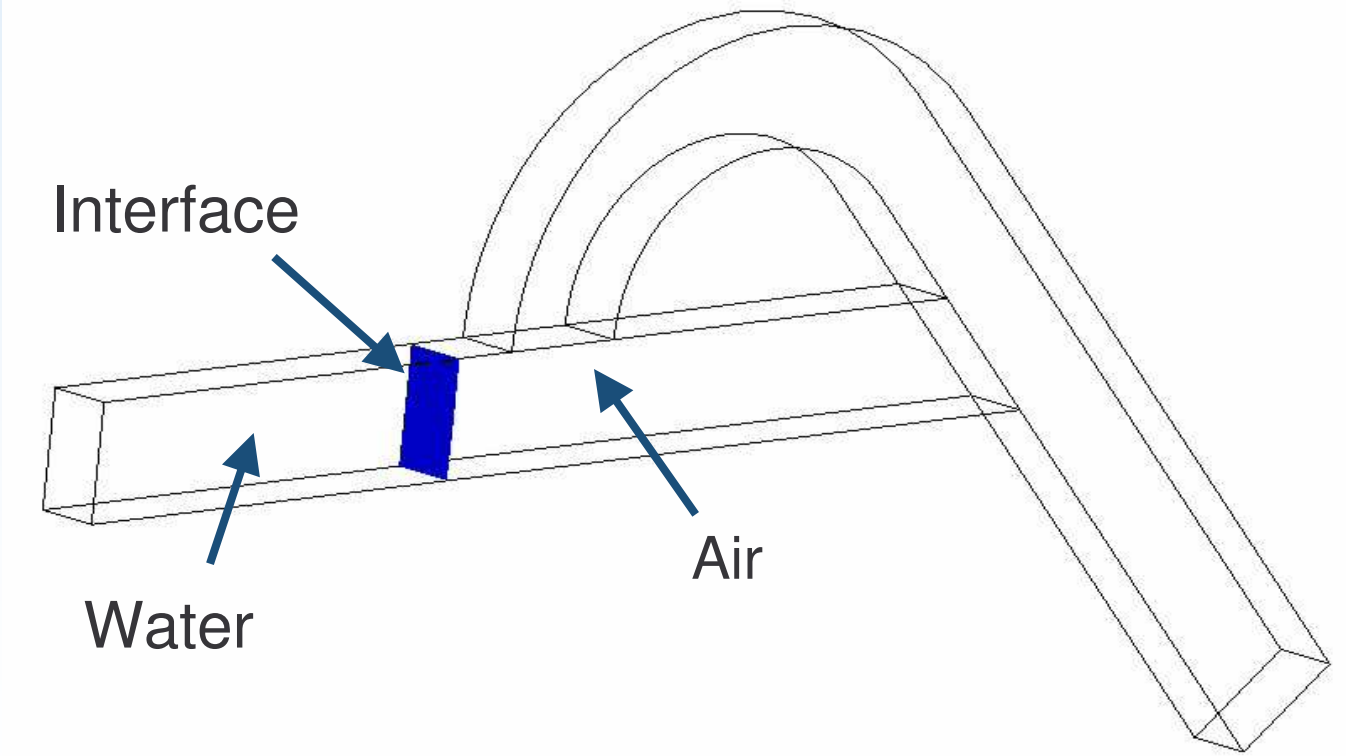
Several Examples of Biochip Modeling

1. Bubble/Particle Filter Design.
2. Particle Manipulation with Dielectrophoresis.
3. DNA Hybridization in a Microchannel.
4. Microbead-Based Immunoassay.
5. **Filling by Wicking.**



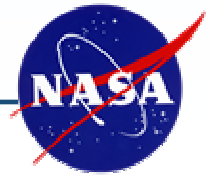
Filling a Tesla Valve by Wicking

- Flow driven entirely by wicking action.
- Inside surface is strongly hydrophilic (0^{o} contact angle).
- Air bubble develops.



What Next?

- Further evaluation of existing biochip technology.
- Improve modeling capabilities and fundamental theory.
- Propose a system-level model by assembling these technologies into a single prototype design.
- Test alternative designs using computer models.
- Work with commercial biochip manufacturers to build an experimental prototype.
- Field test the experimental prototype on the ground and in microgravity.



Thank You